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Nitrogen and sulfide removal from effluent of UASB reactor in a sequencing fed-batch biofilm reactor under intermittent aeration

B.S. Moraes*, J.G.T. Orrú, E. Foresti

Department of Hydraulics and Sanitation, School of Engineering of São Carlos, University of São Paulo, Av. Trabalhador São-Carlense, 400, Centro, 13566-590, São Carlos, SP, Brazil

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ABSTRACT

Simultaneous nitrification/denitrification (SND) coupled with sulfide oxidation may be suitable for the post treatment of effluents from anaerobic reactors. These effluents contain ammonium, which must be nitrified, and sulfide, which could be used as an endogenous electron donor for autotrophic denitrification. The SND process occurred in a sequencing fed-batch biofilm reactor of 8 h cycles, operated under intermittent aeration. The effect of the start-up period and the feeding strategy were evaluated. The previous establishment of nitrification process with subsequent application of sulfide in low concentrations was the best start-up strategy to enable the occurrence of SDN. The fed-batch mode with sulfide application in excess only in the anoxic periods was the best feeding strategy, providing average efficiencies of 85.7% and 53.0% for nitrification and denitrification, respectively. However, the low overall nitrogen removal efficiency and some operational constraints indicated that autotrophic denitrification using sulfide in a single SBR was not suitable for SND under the assayed conditions.

1. Introduction

Biological nitrogen removal in wastewater treatment plants is conventionally achieved in two separate steps: autotrophic nitrification followed by heterotrophic denitrification. However, some studies on single step nitrogen removal, called simultaneous nitrification/denitrification (SND), have been carried out in order to optimize treatment systems. Compared to conventional systems, the costs could also be reduced (Yoo et al., 1999; Canto et al., 2008). In this process, nitrification and denitrification occur concurrently in the same reactor. The advantages of this single step SND process over the conventional two steps process are already known. The space and aeration requirements, and the time necessary to complete nitrification and denitrification reactions are lower if SND is successfully applied (Münch et al., 1996; Yoo et al., 1999).

Sequencing batch reactors (SBR) are normally used to promote the SND process (Mace and Mata-Alvarez, 2002). Basically, they are operated in sequential cycles composed of four stages: feeding, reaction, settling and liquid withdrawal. However, some operational strategies have been studied in order to optimize the performance of these reactors, such as modifications in the feeding mode, the use of immobilized biomass and the application of intermittent aeration (Chen et al., 2000; Ratusznei et al., 2003; Canto et al., 2008). The fed-batch mode is an alternative to keep the substrate concentration in low levels inside the reactor, so that the feeding stage has a longer period than that conventionally adopted. This strategy can improve the electron donors' distribution and, because of dilution, it can also avoid inhibition by the substrate (Tilche et al., 1999; Poo et al., 2004). On the other hand, the use of immobilized biomass on inert support allows the elimination of the settling step and the reduction of the overall cycle time. Additionally, biomass losses are normally very low, resulting in high cellular retention times. According to Pochana and Keller (1999), the SND activity increases in reactors containing large sludge flocs. Thus, this technology seems to be especially advantageous in systems containing immobilized cells. Limitations of oxygen diffusion into the biofilm lead to anoxic conditions in its inner parts, favoring denitrification in this region, while nitrification occurs on the aerated surface of flocs.

Considering the occurrence of denitrification inside the flocs, the SBR for nitrogen removal can be operated under aerated conditions during all periods (Chen et al., 2000). However, the total nitrogen removal efficiency may not be acceptable. This way, the application of intermittent aeration can improve the nitrogen removal efficiency, by incorporating an anoxic period for denitrification. During the period of non-aeration, the reactor operates essentially as an anoxic reactor. In this period, a depletion of dissolved oxygen (DO) concentration occurs so that the concentration of oxidized forms of nitrogen decreases and the concentration of ammonium increases. In the subsequent aeration period, ammonium is oxidized to nitrate or nitrite. The specific times for aeration and

* Corresponding author. Tel.: +55 16 3373 8358.
E-mail addresses: bs_moraes@yahoo.com.br (B.S. Moraes), eforesti@sc.usp.br (E. Foresti).

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non-aeration are essential to the performance of the treatment system.

By targeting the post treatment of effluents from anaerobic reactors, the use of conventional heterotrophic denitrification is disadvantageous due to the low concentration of organic matter in this type of wastewater. Therefore, the application of this process would require the addition of organic electron donors for denitrification. An innovative alternative is the use of sulfide as electron donor for autotrophic denitrification because this compound is commonly present in the effluent of anaerobic reactors. Moreover, such effluents contain ammonium that needs to be oxidized prior to the denitrification step. Thus, the SND process using sulfide for autotrophic denitrification seems to be a viable solution for nitrogen and sulfide removal of effluents from anaerobic reactors. Although there are promising studies on sulfide-oxidizing autotrophic denitrification, this process has not been well described and understood yet (Beristain-Cardoso et al., 2006; Mahmood et al., 2007a; Cervantes et al., 2009; Moraes et al., 2011), especially when associated with SND (Pérez et al., 2007).

According to Moraes et al. (2011), the final products of autotrophic denitrification are influenced by sulfide concentration in the wastewater. When sulfide concentration is in excess to the stoichiometrically required (N/S molar ratio of 1.6 for denitrification via nitrate, and 2.7 for denitrification via nitrite), sulfide oxidation tends to occur partially with the formation of elemental sulfur. In contrast, when sulfide concentration is equivalent to the stoichiometric requirement, complete oxidation to sulfate takes place. Other authors also observed this behavior (Krishnakumar and Manilal, 1999; Beristain-Cardoso et al., 2006; Moraes et al., 2011). Regarding sulfide concentration in effluents from anaerobic reactors, it is dependent on wastewater characteristics. Therefore, the amount of sulfide generated in the anaerobic treatment of domestic sewage is expected to vary according to the composition of this type of wastewater, which is variable according to the socio-economic habits of the contributor population. Thus, the range of sulfide concentration present in the influent may drive different behaviors inside reactors applied to autotrophic denitrification.

This study investigated the feasibility of SND coupled to sulfide oxidation in a sequencing fed-batch biofilm reactor intermittently aerated for the post treatment of effluent from an upflow anaerobic sludge blanket (UASB) reactor. The main objective of the investigation was to evaluate two start-up alternatives and feeding strategies for the establishment of nitrification and denitrification.

2. Methods

2.1. Sequencing fed-batch biofilm reactor

The nitrifying/denitrifying reactor, with a total volume of 7.5 L, was made of borosilicate glass and equipped with a mechanical stirrer composed of two radial flow-impeller turbines of 3.0 cm diameter. A perforated stainless steel basket was allocated inside the reactor to support the biomass immobilized on cubic matrices of polyurethane foam. The basket was in the form of a hollow cylinder and its central region accommodated a shaft for the stirrer. At the top of the reactor, two perforated pipes with 2.0 cm internal diameter were allocated to support micro-sensors for dissolved oxygen (DO) and redox potential (ORP) measurements. These micro-sensors were constructed specifically for the use in the present reactor. They were composed of a sensitive tip of 10–30 μm in diameter, which was supported by a small glass bar (3 mm in diameter and 7 cm long). The micro-sensors were connected to a data acquisition block coupled to a computer. The monitoring data were obtained using software developed by T&S Electronic Equipments. At the bottom of the reactor, porous stones were placed for air dispersion. Aeration was provided by an aquarium air pump. Two pumps were used for filling and discharging the liquid: a peristaltic pump and a diaphragm pump, respectively. The experimental setup is presented in Fig. 1.

2.2. Feed composition and inoculum

The reactor was fed with effluent from an UASB reactor treating synthetic substrate simulating domestic sewage. This effluent was

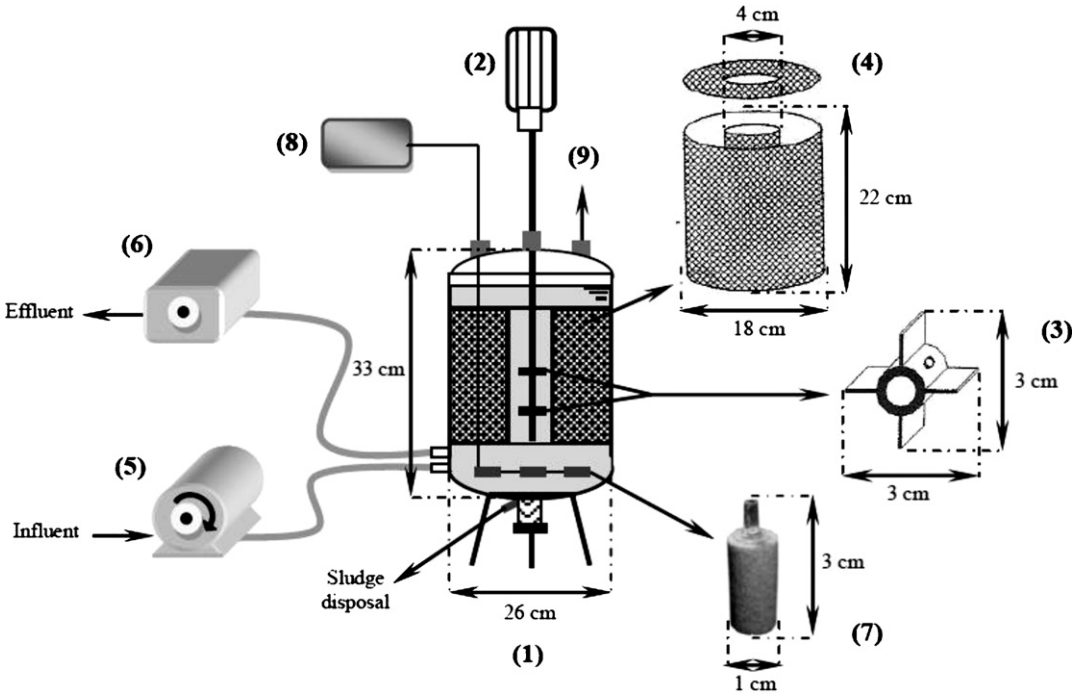

Fig. 1. Schematic representation of the experimental setup: (1) cross-sectional view of the nitrifying/denitrifying reactor operated in fed-batch mode; (2) mechanical stirrer; (3) turbine impeller; (4) stainless steel basket; (5) peristaltic pump-feeding; (6) diaphragm pump-discharge; (7) porous stone; (8) aquarium air pump; (9) entry for micro-sensors.

collected in a 21 L container, stored at 4 °C, and pumped into the nitrifying/denitrifying reactor under fed-batch mode. The average influent ammonium concentration was 40 mg NH_4^+-N L^{-1}. Alkalinity as sodium bicarbonate was added separately at the average concentration of 500 mg $CaCO_3$ L^{-1} to attend the demand for nitrification and to enhance the buffering capacity of the influent. The effluent from the UASB reactor was free of sulfide, which was added separately as sodium sulfide solution to allow a more precise control of the sulfide concentration added to the reactor. This solution was kept in sealed 1 L Duran flask containing a bladder attached to the lid filled with N_2 gas to avoid the chemical oxidation of this compound. Influent sulfide concentration ranged from 0 to 55 mg S L^{-1}. The dissolution of sodium sulfide in the liquid causes the release of OH^- ions and, consequently, the increase of pH of a liquid medium with low buffering capacity. For this reason, alkalinity was added to avoid a large increase of pH influent, which was maintained at the range of 8.5–8.9.

Cubic matrices of polyurethane foam of 1.0 cm edge were used as support for biomass immobilization. Two types of inoculum were evaluated, separately. The first one was obtained from a UASB reactor treating poultry slaughterhouse wastewater. The other one was obtained from an activated sludge system of a car manufacturing company. The granular sludge from the UASB reactor was previously disintegrated in a blender to obtain a homogenous mixture. Then, for both types of sludge evaluated, the cubes of polyurethane foam were mixed and compressed with a large quantity of sludge. Thereafter, they were kept in contact for at least 2 h, as recommended by Zaiat et al. (1994). After this period, the foam matrices were transferred to a sieve, in which the excess of sludge was removed by washing the foam on the screen. Finally, the inoculated foam cubes were placed into the perforated stainless steel basket allocated inside the reactor, until its complete filling.

2.3. Experimental procedures

The reactor was operated in fed-batch mode of 8 h cycles. Each cycle consisted of two anoxic phases intercalated with two aerated phases. The cycles always initiated with an anoxic phase and finished with an aerated phase. Each phase had the time of 116 min and the discharge was performed during the last 16 min of each cycle. The reactor was kept in an incubator under a controlled temperature of 30 ± 1 °C. The stirring was kept at 150 rpm during all the operation period.

Two start-up strategies were tested initially: intermittent aeration (116 min/116 min) with 50 mg L^{-1} of sulfide as electron donor for autotrophic denitrification (strategy I); and the aeration supply during all cycle with no sulfide provision for the previous establishment of nitrification, followed by the interchange of anoxic/aerated (116 min/116 min) periods with low sulfide concentrations (15 mg TDS L^{-1}) in the feed (strategy II). In the first strategy, the inoculum was obtained from the UASB reactor. For the second strategy, sludge from the activated sludge system was used. During the start-up period, the feed step was carried out in fed-batch mode during the first 7 h of the 8 h cycle, for both strategies. The time of the cycles and the initial times of aerated/anoxic phases were defined according to the intrinsic kinetic parameters of sulfide-oxidizing autotrophic denitrification determined by Moraes and Foresti (2012).

After the start-up strategy was defined, the feeding strategy was evaluated. The fed-batch mode during the first 7 h of the cycles (condition 1, A and B) was compared with the fed-batch only during the anoxic phases (intermittent fed-batch: condition 2, A and B). In the former strategy, the reactor was almost completely emptied during the discharge, leaving only 1/7 of the reactor volume. In the latter, 1/3 of the reactor volume was kept with effluent. For both strategies, varying sulfide concentrations were applied, as detailed in Table 1, which presents all operational conditions applied to the reactor during the feed strategies evaluation.

The air flow was kept at 20 L h^{-1} during the start-up and all operation. However, the concentration of DO varied according to the sulfide concentration applied, since this compound causes depletion of DO in the liquid medium.

The behavior of nitrogen and sulfur compounds over the cycles was evaluated by means of temporal profiles of concentration, as well as the behavior of DO and ORP. These profiles were performed at the end of each experimental condition.

2.4. Analytical methods

All chemical analyses were performed according to Standard Methods for the Examination of Water and Wastewater (APHA/AWWA/WEF, 2005). Chemical oxygen demand (COD) was determined by the colorimetric method and bicarbonate alkalinity (BA) by titration. Nitrate (NO_3^--N), nitrite (NO_2^--N) and sulfate (SO_4^{2-}-S) were determined by ion chromatography (ICS-5000, Dionex, USA). Ammonium (NH_4^+-N) was measured by flow injection analysis (FIA) and free ammonia was calculated according to Eq. (1), proposed by Anthonisen et al. (1976). The total dissolved sulfide (TDS) was determined using the methylene blue colorimetric method. DO and ORP were measured using micro-sensors. Nitrogen gas and sulfur intermediary compounds were estimated based on mass conservation principles. Volatile suspended solids (VSS) and attached solids in polyurethane foam were gravimetrically determined.

$$NH_3 - N = \frac{17 \cdot (\text{Ammonium}_{\text{total}} - N) \cdot 10^{pH}}{(14 \cdot Kb/Kw) + 10^{pH}} \quad (1)$$

In which $Kb/Kw = e^{6344/(273+T)}$, NH_3-N is the concentration of free ammonia as N (mg NH_3-N L^{-1}), $\text{Ammonium}_{\text{total}}$-N is the nitrogen concentration as total ammonium (mg N L^{-1}), T is the temperature (°C).

3. Results and discussion

3.1. Start-up strategies

The reactor was operated for 45 days under strategy I (days 1–45). Neither ammonium consumption nor nitrate and/or nitrite formation was detected at any time. Nitrification was inhibited by the presence of sulfide at high concentrations (43.8 ± 9.4 mg TDS L^{-1}), which caused the increase of pH (8.9 ± 0.3). At such high pH, free ammonia (17.0 ± 5.9 mg NH_3-N L^{-1}) was

Table 1
Description of the operational conditions applied after the start-up of the reactor.

Condition	Feed mode	Sulfide concentration (mg TDS L^{-1})	Anoxic phase/ aerated phase (min)	Feed time/total cycle time	Fed volume/total volume treated per cycle
1A	Fed-batch (7 h)	20–25	116/116	7 h/8 h	6.5 L/5.6 L
1B	Fed-batch (7 h)	5–10	116/116	7 h/8 h	6.5 L/5.6 L
2A	Intermittent fed-batch	20–25	116/116	232 min/8 h	6.5 L/4.3 L
2B	Intermittent fed-batch	45–55	116/116	232 min/8 h	6.5 L/4.3 L

formed. According to Anthonisen et al. (1976), concentrations between 10 and 150 mg L^{-1} of free ammonia cause inhibition of ammonia-oxidizing bacteria and thus, of the ammonium oxidation. These results also revealed that nitrifying bacteria present in the inoculum was not able to establish and to adapt to the presence of the toxic compounds – sulfide and free ammonia – in the experimental conditions of this strategy. Even with a longer reactor start-up period for this strategy, no evidence of establishment of nitrification was observed. In addition, the denitrifying characteristic of the anaerobic sludge used in strategy I might have contributed to impair the nitrification process. Thus, the experiment was dismantled and the start-up strategy II was initiated.

Under strategy II (days 1–79), complete nitrification was set in 20 days. However, the nitrogen mass balance could not be closed because the formation of oxidized nitrogen compounds did not correspond to the ammonium consumption (Fig. 2a). Hence, the hypothesis of heterotrophic denitrification inside the foam was considered, since COD consumption (average of 63%) was observed during this period. Considering that the biomass concentration decreased from 0.153 to 0.102 g VSS g foam^{-1}, heterotrophic denitrification via endogenic activity also might have contributed to the reduction of the oxidized nitrogen compounds. Additionally, heterotrophic denitrification produces alkalinity. Therefore, as BA concentration was higher than the amount theoretically calculated based on nitrification (Fig. 2b), the hypothesis of heterotrophic denitrification occurrence was supported by the measurements of this parameter. This period was extended until the exhaustion of denitrification was observed, which occurred from day 28 onwards (Fig. 2a).

After nitrification was established, the reactor was subjected to intermittent aeration with the applying of low sulfide concentrations (11.6 ± 5.8 mg TDS L^{-1}). The reactor was operated during 39 days in this condition and the occurrence of autotrophic denitrification coupled with sulfide oxidation in the SND process was evaluated. Mass balance of nitrogen compounds showed that 4.0 ± 3.1 mg N L^{-1} were denitrified during this period. Total sulfide consumption with sulfate production occurred during this period (13.4 ± 3.8 mg SO_4^{2-}-S L^{-1}). Effluent nitrite and nitrate concentrations were 13.1 ± 2.3 and 10.7 ± 1.9 mg N L^{-1}, respectively. However, sulfide caused toxicity on nitrification, since the average efficiency of this process decreased from 97 ± 8% to 77 ± 4%. During the whole period, nitrifying bacteria showed no signs of increase in its biological activity so that nitrification efficiency remained constant. Thus, it was supposed that nitrifying microorganisms present in the inoculum, which was not essentially nitrifying, were not able to adapt to the presence of sulfide. Based on these results, the next operation conditions aimed to improve autotrophic denitrification by providing a greater availability of the electron donor without inhibiting the nitrification process.

3.2. Sequencing fed-batch biofilm reactor operation

After defining strategy II for the reactor start-up, the operation continued for over 102 days, totalling 180 days of operation, including the period of strategy II of reactor start-up (78 days). In this section, only the results obtained from day 80 onwards are shown. The average behavior of the nitrogen compounds is presented in Fig. 3. The average values of the other monitored parameters are presented in Table 2, divided according to the evaluated conditions. In condition 1A (days 80–115; Fig. 3a), the increase of sulfide concentration in relation to the previous period resulted in a reduction of nitrification efficiency, which dropped sharply to the average value of 42.3%. In this case, sulfide significantly inhibited the action of ammonia-oxidizing bacteria. This result is in accordance to Sears et al. (2004), which reported complete inhibition of ammonia oxidation in the presence of 0.5 mg TSD L^{-1}, reinforcing the extreme sensibility of ammonia-oxidizing bacteria to soluble sulfide. On the other hand, Erguder et al. (2008) observed much lower inhibition effects on a nitrifying sequential batch reactor (2 days cycles) subjected to sulfide pulses (45 mg S L^{-1}) during anoxic conditions. The authors reported that nitrite-oxidizing bacteria were more sensitive to sulfide than the ammonia oxidizers. However, the inoculum consisted of a dense nitrifying suspension and the ammonium influent concentration was much higher (680 mg NH_4^+-N L^{-1}) than in the present work, facts that probably contributed to higher ammonia oxidizers activity and to its better adaptation to the presence of sulfide. In addition, unlike the present research, the pH was kept at 7.5 ± 0.2, which prevented the formation of free ammonia. Thus, sulfide inhibition seems to be more related to the raise of pH than DO depletion because sulfide concentration used by the cited authors was much higher than the one presented in this work. Additionally, Joye and Hollibaugh (1995) also detected that DO concentration did not differ significantly between experiments with sulfide addition and control experiments (without sulfide). These authors evaluated the influence of sulfide inhibition on nitrification in estuarine sediments. However, nitrification rates decreased significantly with sulfide addition, even at low concentrations (1.9 mg HS^- L^{-1}).

On the other hand, the increase of sulfide in this work caused DO depletion from 3.0 mg L^{-1} during the period of nitrification establishment (without sulfide) to 2.5 mg L^{-1} in condition 1A. These DO values corresponded to the maximum measured value in the aerated periods from temporal profiles (data not shown). The inhibition caused by DO insufficiency is commonly reported at values

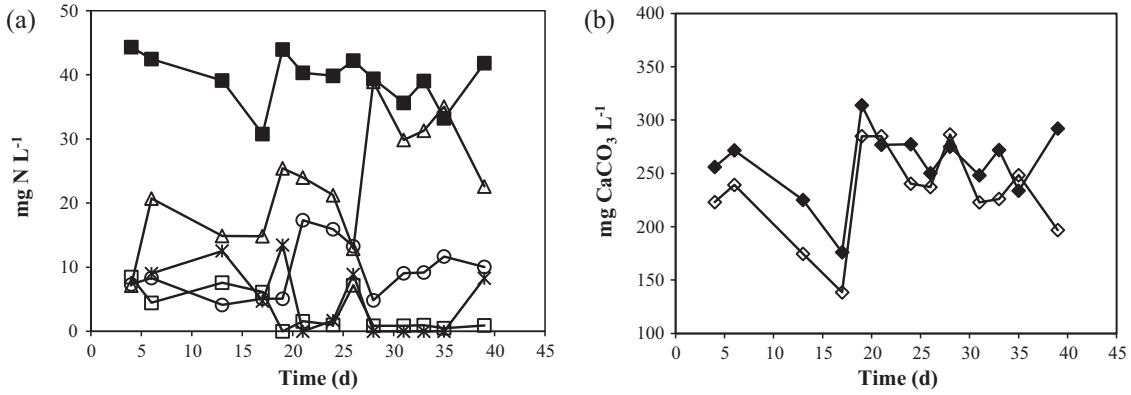

Fig. 2. Monitoring of (a) nitrogen compounds and (b) bicarbonate alkalinity during the establishment of nitrification (strategy II): (■) ammonium-N influent; (□) ammonium-N effluent; (△) nitrate-N; (○) nitrite-N; (ж) calculated N_2-N; (◊) measured BA consumption; (♦) theoretical BA consumption.

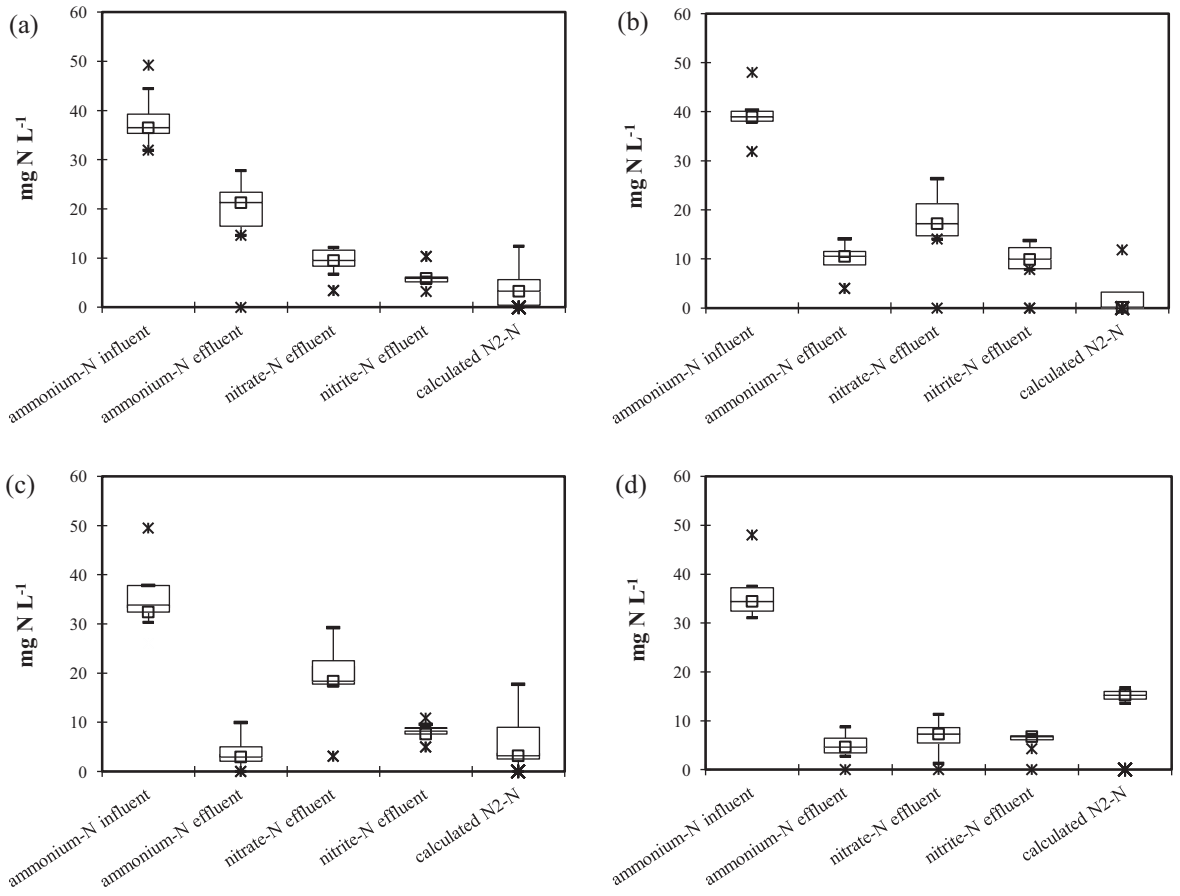

Fig. 3. Concentration of nitrogen compounds at each monitored condition: (a) 1A (days 80–115); (b) 1B (days 116–134); (c) 2A (days 135–154); (d) 2B (days 155–180). Data include inter-quartile deviation and median (larger box), (□) average value, (ж) outlier and (—) maximum (upper whisker) and minimum (lower whisker) limits of non-discrepant values.

lower than 0.5 mg L^{-1}, inhibiting mainly the nitrite oxidizers and promoting nitrite accumulation (Schmidt et al., 2003; Erguder et al., 2008). Thus, in this work DO depletion was not the limiting factor to the occurrence of nitrification.

The temporal profile of sulfur compounds show the sulfate production and very low sulfide availability during the whole cycle (Fig. 4). It can be seen that a small amount of sulfide was available for autotrophic denitrification, which could explain the low values of calculated N_2-N. Such unavailability of sulfide probably occurred as a result of the aeration inside the reactor, which promoted the chemical oxidation of sulfide mainly to sulfate and/or provided the oxygen as preferential electron acceptor by microorganisms. According to Buisman et al. (1990), biological oxidation of sulfide with oxygen is significantly faster than the chemical non-catalyzed oxidation of sulfide with oxygen. Thus, biological sulfide oxidation with oxygen probably took place inside the reactor, since sulfide was not significantly detected even during the anoxic periods. Besides, some of the species of chemolithotrophic colorless sulfur bacteria, which promote autotrophic denitrification, such as *Thiobacillus* sp., are facultative and can use oxygen as electron acceptor instead of oxidized nitrogen compounds (Kuenen, 1975). The applied feeding strategy included the discharge of 6/7 of the reactor volume at the end of the cycles. Therefore, the availability of nitrate/nitrite was low at the beginning of each cycle, since the

Table 2
Average values of the parameters monitored during the operational period, divided into the evaluated conditions.

Condition	Operation period (day)	Influent				
		COD (mg L^{-1})	BA (mg $CaCO_3$ L^{-1})	pH	TDS (mg S L^{-1})	SO_4^{2-} (mg S L^{-1})
1A	80–115	77.4 ± 7.4	796.1 ± 98.7	8.5 ± 0.1	23.6 ± 7.2	8.3 ± 1.7
1B	116–134	83.3 ± 9.1	702.8 ± 41.7	8.1 ± 0.3	6.6 ± 3.6	10.0 ± 9.2
2A	135–154	73.4 ± 11.2	684.6 ± 39.0	8.4 ± 0.1	20.2 ± 5.5	5.0 ± 3.4
2B	155–180	79.8 ± 3.1	735.9 ± 12.8	8.9 ± 0.0	53.3 ± 2.9	8.6 ± 1.4
Condition	Operation period (day)	Effluent				
		COD (mg L^{-1})	BA (mg $CaCO_3$ L^{-1})	pH	TDS (mg S L^{-1})	SO_4^{2-} (mg S L^{-1})
1A	80–115	24.4 ± 8.3	657.0 ± 94.1	8.1 ± 0.1	0.0 ± 0.1	28.2 ± 7.2
1B	116–134	20.1 ± 3.7	450.5 ± 102.9	7.8 ± 0.2	0.0 ± 0.1	21.9 ± 7.5
2A	135–154	22.3 ± 15.0	388.6 ± 42.9	8.0 ± 0.1	0.0 ± 0.1	20.0 ± 5.2
2B	155–180	26.4 ± 6.5	346.4 ± 70.2	8.0 ± 0.3	0.0 ± 0.1	47.8 ± 8.4

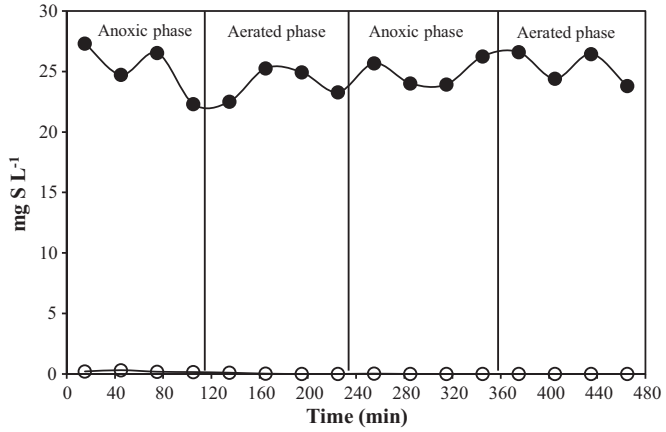

Fig. 4. Temporal profile of sulfur compounds during an 8 h cycle for condition 1A under steady-state condition: (○) sulfide and (●) sulfate.

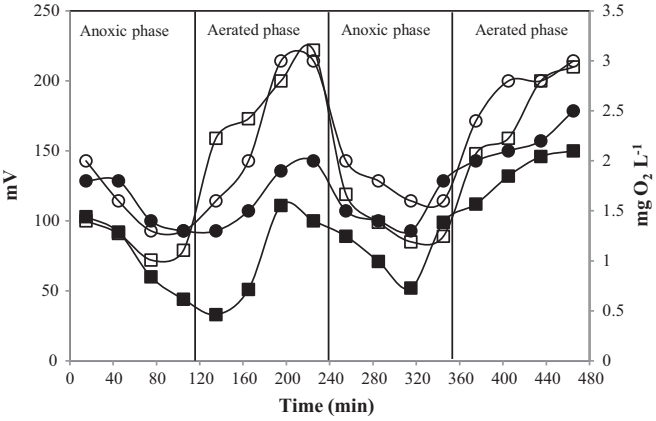

Fig. 5. Comparison of temporal profiles during an 8 h cycle under steady state between the evaluated feeding strategies: condition 1A – (■) ORP and (●) DO; condition 2A – (□) ORP and (○) DO.

effluent from the UASB used as the feed contained only nitrogen in ammonium form. This way, the denitrifying facultative bacteria probably used oxygen for sulfide oxidation, at least in the first anoxic phase of the cycles.

Considering these results, the feeding strategy would have to be amended in order to allow better availability of sulfide to autotrophic denitrifying bacteria. Allied to this, such strategy would also have to lower as much as possible the toxic effect of sulfide on nitrification. Therefore, the intermittent feeding only during the anoxic periods and the partial discharge of the reactor volume at the end of the cycles seemed to be the solution (condition 2, A and B).

Before changing the feeding strategy, the nitrification recovery was evaluated by reducing the sulfide concentration, which corresponded to condition 1B (days 116–134). The sulfide addition was not suppressed, in an attempt to adapt the nitrifying microorganisms to the presence of this toxic compound. The results showed that nitrification was recovered, achieving an average efficiency of 72.3%. The main product of nitrification was nitrate (Fig. 3b). Thus, nitrite-oxidizing bacteria, considered more sensitive to soluble sulfide than ammonia-oxidizing bacteria (Bentzen et al., 1995; Bae et al., 2002; Erguder et al., 2008), were also recovered. As described by Sears et al. (2004), these results showed that the inhibition of sulfide on nitrification is reversible by reducing the concentration of the toxic compound. Although nitrification has been recovered, its recovery was not complete. It is worth mentioning that sulfide was always present during all the aerated batch stages from the end of the start-up period of strategy II up to the condition 1B (95 days). Along this time, nitrification was never satisfactory (lower than 81%). This result reinforces the supposition that nitrifying bacteria present in the inoculum were never adapted completely to the presence of sulfide.

Regarding denitrification, in this condition, it was practically non-existent due to the insufficiency of electrons for the reduction of oxidized nitrogen compounds (Fig. 3b). As in the previous condition (1A: days 80–115), such insufficiency was resulted by the chemical oxidation of sulfide and/or the biological use of oxygen as the preferential electron acceptor.

When the reactor began to be operated under condition 2A (days 135–154), the denitrification started to occur in an unstable way: $3.7 \pm 6.5\ mg\ N\ L^{-1}$ were denitrified (Fig. 3c). It is noteworthy that the concentration of the electron donor was below the value required by stoichiometry based on biochemical reactions. Therefore, the amount of electrons available was not sufficient for the occurrence of complete denitrification. Considering the autotrophic denitrification using nitrate as electron acceptor, which was the main product of nitrification, approximately $50\ mg\ L^{-1}$ of soluble sulfide would be needed. This value is based on Eq. (2), proposed by Mahmood et al. (2007b), and takes into account the average consumption of ammonium in this period. Nevertheless, the autotrophic denitrifying bacteria did not use the total available sulfide ($20.2 \pm 5.5\ mg\ TDS\ L^{-1}$) measured in the feeding to reduce nitrate, since approximately $14\ mg\ N\ L^{-1}$ should have been denitrified if this happened, considering the complete sulfide oxidation to sulfate. In this way, the use of oxygen as the preferential electron acceptor by the microorganisms was still occurring.

$$5HS^- + 8NO_3{}^- + 3H^+ \rightarrow 5SO_4{}^{2-} + 4N_2 + 4H_2O \quad (2)$$

Regarding nitrification, the intermittent feeding strategy was effective to reduce the toxic effect of sulfide on nitrifying bacteria, because the consumption of ammonium increased to 91%. The temporal profile of ORP of one cycle for condition 2A (days 135–154) showed higher oxidation in the medium during the aerated periods when compared with condition 1A (days 80–115), with the same sulfide concentration in the feed (Fig. 5). This feeding strategy avoided the presence of sulfide – a strong reducing agent – during aerated periods, ensuring a more oxidized environment for nitrification. According to Wanner (1991), the optimal ORP value for nitrification is between 100 and 300 mV. This range occurred only for condition 2A (days 135–154). In addition, the intermittent feeding promoted the decrease of free ammonia during the aerated periods to values lower than $1.6\ mg\ NH_3\text{-}N\ L^{-1}$ (Fig. 6b). This value is close to the lower limit of inhibitory concentrations of free ammonia for nitrite-oxidizing bacteria presented by Abeling and Seyfried (1992), ranging from1 to $5\ mg\ NH_3\text{-}N\ L^{-1}$. In accordance to this information, nitrite accumulation was lower than 3.1 mg $NO_2{}^-\text{-}N\ L^{-1}$, with most of the ammonium being oxidized to nitrate. On the other hand, in condition 1A (days 80–115), free ammonia was up to $5\ mg\ NH_3\text{-}N\ L^{-1}$ and nitrite accumulation was up to 9 mg $NO_2{}^-\text{-}N\ L^{-1}$ (Fig. 6a).

Attempting to improve the performance of denitrification, the concentration of the electron donor was increased (condition 2B: days 155–180). Considering the nitrified ammonium-N ($27.5 \pm 4.6\ mg\ NH_4{}^+\text{-}N\ L^{-1}$), the applied sulfide concentration ($53.3 \pm 2.9\ mg\ TDS\ L^{-1}$) was slightly in excess relative to the electron acceptors. This measure was taken to provide electron donor concentration sufficient to autotrophic denitrification. If this had not been done, autotrophic denitrification would remain incomplete due to limiting sulfide concentrations, even if the sulfide oxidation by oxygen had not been occurred. The efficiency of denitrification enhanced to an average of 53.0%, while the efficiency of nitrification decreased to 85.7% (Fig. 3d). A decrease of nitrate

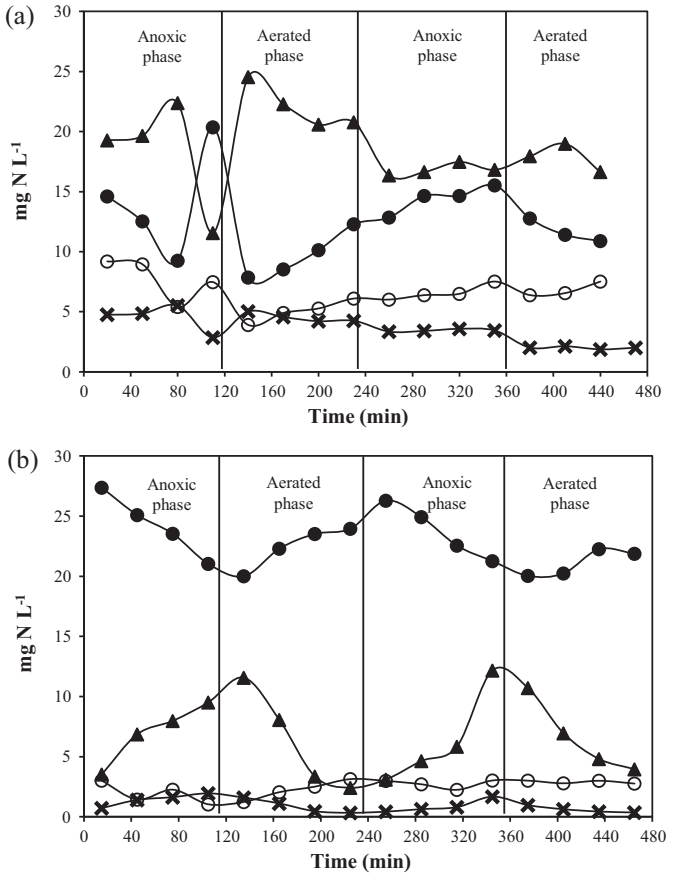

Fig. 6. Behavior of nitrogen compounds during the temporal profile of an 8 h cycle under steady state for (a) condition 1A and (b) condition 2A: (▲) NH_4^+-N; (×) NH_3-N; (●) NO_3^--N; (○) NO_2^--N.

and nitrite concentrations in the effluent was observed. As presented in Fig. 7, the ORP profile of an 8 h cycle under steady state shows favorable values for nitrification and denitrification during the aerated and anoxic phases, respectively. According to Wanner (1991), the optimal ORP values for denitrification are in the range of −50 to 50 mV. It is noteworthy that the optimum ORP values for denitrification were observed only at the middle of the anoxic periods. Thus, the favorable environment for denitrifying process was not obtained during the entire anoxic period, i.e., the duration of anoxic phases may not have been enough to allow the complete occurrence of denitrification.

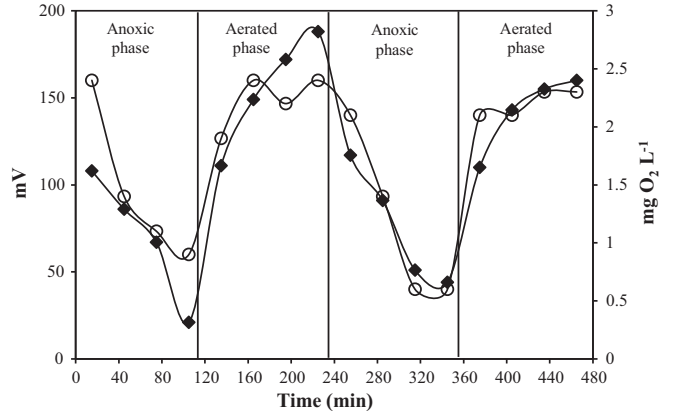

Fig. 7. Temporal profiles of (♦) ORP and (○) DO concentration during an 8 h cycle under steady state for condition 2B.

Differently from the other conditions, partial sulfide oxidation occurred in condition 2B (days 155–180), because the accumulation of a whitish layer at the bottom of the reactor was observed, which is characteristic of the presence of elemental sulfur. Several authors also reported the accumulation of elemental sulfur in denitrifying reactors, when the sulfide concentration was in excess relatively to the required by the stoichiometry (Krishnakumar and Manilal, 1999; Reyes-Avila et al., 2004; Beristain-Cardoso et al., 2006). The results of BA revealed the use of the elemental sulfur previously formed as electron donor for autotrophic denitrification. Effluent BA values were lower than expected when compared to the other conditions (Table 2). Unlike the denitrification from sulfide oxidation, the use of elemental sulfur as electron donor promotes the consumption of alkalinity in the medium, as described by Koenig and Liu (2002). According to the authors, the use of elemental sulfur in autotrophic denitrification consumes about 4 g $CaCO_3$ L^{-1} per g NO_3^--N L^{-1}. Hence, the autotrophic denitrification was not effective in returning the alkalinity in the medium for the nitrification of the subsequent phase, requiring the addition of external alkalinity to the occurrence of both processes. Pérez et al. (2007) also reported the need for complementary addition of bicarbonate to control the pH in the desired range because of the incomplete half alkalinity recovery in the denitrification periods. The authors operated a SBR to promote SND process and showed the feasibility of using sulfide to develop autotrophic denitrification. However, the accumulation of 30 mg NO_2^--N L^{-1} was detected due to the low concentrations of sulfide applied, equivalent to 80–85% of the stoichiometrically required. In this case, the authors reported the necessity to recirculate the effluent from SBR to the head plant to remove this compound. Thus, complete nitrogen removal was not obtained in the single SBR in the conditions assayed, being necessary a complementary treatment to remove nitrite from the effluent.

Based on the obtained results, SND coupled to sulfide oxidation in a single SBR does not seem to be suitable, unless additional treatment is applied to the effluent from that reactor. At the best operational conditions for autotrophic denitrification, a decrease or even the inhibition of nitrification activity occurred. In addition, for the occurrence of both processes in a single reactor, a large and accurate operational control regarding sulfide concentration is required. It is clear that sulfide, even at low concentrations, is an important inhibitor to the nitrifying process. In this way, the applying of such configuration, in large scale, for the post-treatment of effluents from anaerobic reactors is very restrictive. The treatment system would need a strict control of sulfide concentrations contained in the effluent, in order to avoid the inhibition of nitrifying bacteria.

4. Conclusions

The start-up of the reactor was an important factor to the establishment of SND coupled to sulfide oxidation in SBR. The previous establishment of nitrification and the maintenance of low sulfide concentrations was the key to allowing the development of nitrifying activity, which is strongly sensitive to the presence of sulfide. On the other hand, the feeding strategy could improve the performance of autotrophic denitrification in the SBR subjected to intermittent aeration. The intermittent fed-batch only in the anoxic periods improved the overall efficiency of nitrogen and sulfide removal. However, the most favorable condition for autotrophic denitrification promoted a decrease in the nitrification efficiency. Additionally, there was accumulation of elemental sulfur and large consumption of alkalinity that was not produced as expected in denitrification. Based on the obtained results, SND coupled to sulfide oxidation in a single SBR intermittently aerated was

not satisfactory for the post treatment of domestic effluents from UASB reactors under assayed conditions. Such application seems to be restrictive in treatment plants due to the operational difficulty in establishing both processes in the same reactor. Thus, different reactor configurations should be more appropriate for the viability of SND using sulfide as electron donor.

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